STUDIES ON HISTOPATHOLOGY OF SOME ROOT-KNOT NEMATODE INFECTED CUCURBITS

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بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِيْمِ

Proclaim! And thy Lord.
Is Most Bountiful.
He Who taught.
(The use of) the pen,
Taught, man that.
Which he knew not.

TO MY EVERLOVING PARENTS

Dr. Ziauddin A. Siddiqui
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C E R T I F I C A T E

Certified that the work embodied in this dissertation entitled "STUDIES ON HISTOPATHOLOGY OF SOME ROOT-KNOT NEMATODE INFECTED CUCURBITS" is the bona-fide work carried out by Mr. Hisamuddin under my supervision and is suitable for submission for the M.Phil Degree of Aligarh Muslim University, Aligarh.

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H1 SAMUDDIN

C O N T E N T S

1. Introduction

INTRODUCTION

In plants, nematode activities result in morphological and physiological changes of the affected tissues. These changes may be destructive, adaptive or neoplastic. In destructive changes, the nematodes, during feeding, cause damage or death of the cells by removing their contents rapidly or slowly but completely. In adaptive changes the host cells adapt to nematodes by enlarging and by increasing their metabolic activities. In neoplastic changes the cells undergo growth and multiplication. Various types of galls associated with certain nematodes are examples of neoplasm (Dropkin, 1980).

All phytoparasitic nematodes remove cell contents through their stylets, but each kind of nematode has its own way of feeding, and each induces its own type of tissue damage. All plant - parasitic nematodes are classified into two broad groups ectoparasites and endoparasites depending upon their feeding sites and mode of feeding. Ectoparasites are further of two types surface feeders and subsurface feeders. The former feed on cells of the root epidermis and on root hairs, e.g., Trichodorus, Paratrichodorus, Tylenchorhynchus etc. of which Trichodorus is the most damaging nematode of this group. The latter feed on cells in the cortex or close to stele by penetrating their stylets and sometimes with

a small portion of the anterior body. This group includes Belonolaimus, Hoplolaimus, Longidorus, Xiphinema etc. Three genera of this group (Hemicycliophora, Longidorus and Xiphinema) induce galls with enlarged and proliferated cells. Endoparasites are also of two types, migratory endoparasites and sedentary endoparasites. Certain migratory endoparasitic nematodes feed on roots of herbaceous plants like Radopholus, Hirschmaniella, Pratylenchus. Radopholus similis makes extensive tunnels through the cortex of banana roots and rhizomes. Certain other feed on aerial parts of herbaceous plants like Aphelenchoides, D. dipsaci etc. Aphelenchoides damage, severely, buds and leaves of strawberries, chrysanthemums, begonias. Aphelenchoides infecting chrysanthemum leaves crawl through stomata into mesophyll tissues where they move about actively in the interveinal areas. Ditylenchus dipsaci induces even more extensive damage in non-vascular tissues of stems, leaves, and cotyledons. Some of the migratory endoparasitic nematodes feed on woody trees e.g., Rhadinaphelenchus invades parenchyma tissue of trunk and roots of coconut and other palms. Sessile endoparasites or sedentary endoparasites may either be partly embedded in the tissue and called as semiendoparasites comprise Tylenchulus semipenetras and Rotylenchulus reniformis, or be entirely embedded like Nacobbus, Heterodera Globodera, Meloidogyne. Tylenchulus semipenetrans and Rotylenchulus reniformis penetrate their anterior portion of the body into the roots and feed on

a set of normal sized cells. Nacobbus invades the stele and stimulates both cell division and the fusion of cell protoplast after partial dissolution of cell walls. A spindle-shaped mass of cells with partially dissolved walls results and the nematode feeds at one end of this group of interconnected cells. The feeding site is at the centre of a conspicuous gall formed by the cell division in cortex and vascular tissues. Heterodera induces cortical cells to enlarge and the walls between them to break down in part. The resulting syncytium invades the stele. Syncytium walls next to xylem vessels develop finger like ingrowths, and the central vacuoles disappear. Nuclei of incorporated cells enlarge but do not divide (Dropkin, 1980). Meloidogyne species usually cause galling of the host tissues in the roots to and occasionally in stems and leaves. Galling is due partly to hyperplasia and hypertrophy of the root cortex and partly to the development of giant cells on which the nematodes feed. The giant cells develop from undifferentiated cells, usually of pericycle, in response to feeding by the nematodes. The giant cells are multinucleate and have irregularly thickened walls (Jones and Northcote, 1972). Usually four to six cells are associated with each nematode and their functioning appears to depend on continuous stimulation by the nematode (Bird, 1962). Heavy galling of plants results in stunting of top growth and relatively greater root to shoot ratio. Infested plants show increased susceptibility to water stress and there is increased systhesis of proteins in the galls with disturbance of the normal

transport of substances between root and shoot.

Meloidogyne interferes with the physiological functions of the plants. Rate of respiration is altered depending upon the host specis and physiological age of the host. Due to disruption of vascular tissue, flow of nutrients and water is affected. Heavy infestation of Meloidogyne in the root decreases rate of photosynthesis in the leaves, considerably. All these anatomical and physiological abnormalities lead to stunting, chlorosis and ultimately death of the plants.

2. Host-Parasite Relationship

HOST-PARASITE RELATIONSHIP

The phytoparasitic nematodes are of two broad types, ectoparasites and endoparasites. Ectoparasites feed at or near plant surfaces and complete their life cycle in the soil while endoparasites embed their entire bodies within plant tissues and feed within. Endoparasites pass their entire life cycle in root tissue except for a brief period when parasitic stage larvae may remain in the soil after hatching. Some of the nematodes have been observed with upto one-third of the body embedded in the root and the remainder of the body in the soil, a relationship sometimes termed semiendoparasitism. However, a distinct division between ectoparasitism and semiendoparasitism does not always exist, as shown, for instance by the feeding of Amplimerlinus spp. (Bridge and Hague, 1974). Development of modes of parasitism within the Tylenchida has been proposed in a sequence from ectoparasites to semiendoparasites to endoparasites by Paramonov (1967); Maggenti (1971).

Depending upon their mobility during parasitism, the nematodes are further divided into migratory and sedentary groups. Migratory nematodes, whether ectoparasites or endo-parasites, move from place to place throughout their life cycle. Some nematodes, such as females of Heterodera and Meloidogyne enter the roots as migratory larvae. Their body swells to nearly spherical and the migration is

no longer possible. They pass their remainder life cycle in a single feeding site. These nematodes are termed sedentary endoparasites.

3. Histopathological Changes

HISTOPATHOLOGICAL CHANGES

Phytoparasitic nematodes feeding on below ground plant parts cause injuries of various levels to the root systems. The tissue or the plant part on which they feed, induce various anatomical and physiological changes leading to the development of specific and characteristic symptoms.

Some migratory ectoparasites those feed on root hairs epidermal cells in a rather cursory manner do not markedly affect the root morphology. Another group of migratory ectoparasites cause death of epidermal cells and the outermost layers of cortical cells. Death of cells is probably due to certain enzymes which are secreted into the host by the parasite. Some of the migratory endoparasites cause surface lesions. When lesions are formed, they frequently coalesce to give root systems a generally discolored appearance. Formation of lesions result from the production of phenolic compounds by enzymes secreted by the nematode. Much more complex, however, are the host-parasite relationships of the sedentary endoparasites which cause hypertrophy and hyperplasia of the stelar parenchyma (Dropkin, 1955; Mountain, 1960).

3.1. ECTOPARASITES

All ectoparasitic nematodes remove cell contents of the surface tissue through their stylets, but each kind of nematode has its own way of feeding, and each induces its own type of tissue damage. Christie and Perry (1951) were the first to establish an experimental evidence for direct damage to plants by an ectoparasitic nematode. The ectoparasites included in this text are : Trichodorus, Tylenchorhynchus, Belonolaimus, Longidorus, Xiphinema, Hemicycliophora, Criconemoides, Hoplolaimus, Helicotylenchus and Rotylenchus .

3.1.1. Trichodorus Cobb, 1913.

Rohde and Jenkins (1957) found that "stubby root" nematode, Trichodorus christie affected tomato roots lack a definite root cap, a reduced meristematic region and the presence of protoxylem near the root apex. It caused maturation of apical meristem in grape-fruit (Citrus paradisi) roots (Standifer, 1960). Root tips are, in many cases, the favourite feeding sites of Trichodorus spp. (Zuckerman, 1961; Russel and Perry, 1966; Alhassan and Hollis, 1966; Wyss, 1971 a, b, c; Högger 1971; Schilt and Cohn, 1975; Stirling, 1976). There was a distinct superficial discoloration of epi-, hypo - and a few outer cells of cortical parenchyma changing from yellow through orange to brown in apple roots attacked by

T. Viruliferus and the aggregation of nematodes was in the zone between the apical meristem and the beginning of the root hair zone (Pitcher, 1967).

3.1.2. Tylenchorhynchus Cobb, 1913.

Earlier studies of 'stunt nematodes' Tylenchorhynchus did not show histological changes as a result of their feeding. Southey (1982) states that all stages of T. dubius feed actively on epidermal cells of roots and root hairs. Cell function is affected and roots of some crops can be discoloured light to dark brown after feeding. According to Sutherland and Adams (1964) the only apparent damage to the roots of red pine parasitized by T. claytoni was a disarrangement of the organisation of the root cap cells resulting from the feeding activity. Nematodes which fed on non-apical portions of the root caused no visible damage. Wyss (1973) found that after feeding of T. dubius on epidermal cells and root hairs of Brassica rapa var. silvestris, the cyclosis eventually stopped and then the cytoplasm coagulated. Root hairs were killed but the growth of the root was not greatly affected even when several nematodes fed close together on epidermal cells in the root hair region.

3.1.3. Belonolaimus Steiner, 1949.

Belonolaimus is commonly known as sting nematode. Owens (1951) found minute necrotic lesions along the tap root of peanuts infected with B. gracilis, as a result of feeding of the nematode. Standifer and Perry (1960) observed lesions on grapefruit (Citrus paradisi) roots consisting of a cavity surrounded by affected cells which were characterized by ruptured cell walls and coagulated protoplasm. Xylem and phloem were found close to the root tip, the root cap was reduced to a few cells in depth, and branch roots arose very close to the apex. All of these observations indicated a general maturation of the entire root tip.

3.1.4 Longidorus Micoletzky, 1922

Feeding by 'needle nematode Longidorus spp. was invariably at or just behind, root tips and caused a characteristic root tip swelling or galling associated with a general reduction of the root system. The meristematic activity was found to be disturbed; root elongation was halted but not cell maturation so that vascular tissue extended close to the apex of the root. Root tip swelling were caused by L. africanus, resulting from hyperplasia of the cortical parenchyma (Cohn & Orion, 1970; Wyss 1970). Root tip galls of chicory, caused by L. apulus, showed hyperplasia in the cortex and

hypertrophy in cambial cells, but root tip galls of celery only contained hypertrophied cells (Bleve-Zacheo et al., 1977). The syncytia in these galls resulted from the break down of cell walls. They were later called "lysogenous cavities" (Bleve-Zacheo et al., 1979). The cytoplasm of these cavities consisted of an amorphous mass in which structures or organelles were no longer detectable, and therefore, the "syncytia" could not be compared, at least in their function, to those induced by sedentary endoparasitic tylenchids (Bleve-Zacheo et al., 1979). Griffiths and Robertson (1984) studied the histochemical changes occuring during the life span of root tip galls on Lolium perenne induced by L. elongatus in different stages. In the intial stage hypertrophy occurred and the cells contained enlarged nuclei and nucleoli, a greater proportion of cytoplasm, and increased concentration of protein. This was followed by hyperplasia; cells divided to give two or four daughter cells, accompanied by a proportionate reduction in volumes of cytoplasm, nuclei, and nucleoli and reduced concentration of RNA and protein. The third stage was secondary hypertrophy with enlarged, amoeboid nuclei and nucleoli and a significant increase in concentrations of RNA and protein. In the final two stages, as feeding by L. elongatus progressively removed cell contents, most cells were devoid of inclusions and galls collapsed and were invaded by soil bacteria.

3.1.5. Xiphinema Cobb, 1913.

Schindler (1957) demonstrated severe stunting of top growth as well as galling of root tips in roses. Davis and Jenkins (1960) while investigating the histology of the galls caused by X. diversicaudatum on rose roots found that galls were formed by a hyperplastic response of cortical tissue. Some hypertrophy occurred, with cortical cells two to three times larger than normal and with a granular-appearing cytoplasm. These cells were somewhat similar to giant cells except that they were smaller and even had multiple (two to three), hypertrophied nuclei. Meristematic activity of the root tips was retarded to varying degrees. In some roots vascular differentiation ranged from the usual lack of development to full development.

While studying host range and pathogenicity of X. index, Radewald and Raski (1962), observed cellular hypertrophy, necrosis and multinucleate condition of undifferentiated cortical cells near the nematode feeding sites. The cortex of infected roots of plants by Xiphinema spp., disintegrated in several areas. Pitcher and Ponsette (1963) found stylet of X. diversicaudatum apparently passing through the vascular tissue and coming to rest with its tip near the outer wall of a sieve tube in Petunia hybrida. Injury to cortical parenchyma was obvious on both sides of the stele and one of the protoxylem elements appeared slightly abnormal.

X. index attacked roots of grapevine turned brown and swelled at tips. Epidermal and outer cortical cells collapsed at feeding sites and showed necrosis. In growing roots multinucleate cells, which were considerably enlarged and contained dense cytoplasm, were formed beneath the layer of necrotic cells (Weischer & Wyss, 1976). Roots of grapevine, healthy and attacked by X. index were studied. First visible symptoms of nematode feeding were hypertrophy of cells and a drastic reduction of cell divisions. At feeding sites multinucleate giant cells and mononucleate hypertrophied cells developed. The walls of these cells showed enlarged pit field. Incomplete cell separation and irregular wall formation were found in giant cells (Rumpenhorst and Weischer, 1981).Griffiths and Robertson (1984) observed that feeding of X. diversicaudatum on strawberry caused a progressive increase in DNA content and size of nuclei. Multinucleate cells were present after 2 and 4 days feeding.

3.1.6. Hemicycliophora de Man, 1921.

Galling by sheath nematodes, Hemicycliophora is caused due to the initiation of cell division in the infection site. The stylet may be inserted from six to eight cells into the cortex, hyperplasia occuring in this area. Hypertrophied nuclei occur in cells about the inserted stylet, but no necrosis has been observed. Van Gundy and Rackham (1961) observed in rough lemon that the

areas in the immediate vicinity of the nematode stylet appeared to be undergoing cell division, giving a dense pattern of small cells, as compared with the larger cells surrounding the area. The nuclei were more deeply stained and larger than those in adja - cent areas. Feeding of Hemicycliophora on tomato roots on water agar in Petri dishes exhibited inhibition of cell elongation initially, evidently due to lack of cell differentiation and maturation. Then hyperplasia of the cortex in the feeding sites formed the root swellings. When the nematodes were removed the swelling stopped and one or more branch roots originated from the swelling.

In cranberry, feeding zone of H. similis was probably limited by the secondary growth of the roots and the formation of cortex tissue. These changes prevented the stylet from penetrating the vascular tissue (Zuckerman, 1961).

H. similis fed on eleven of the thirteen plants near the root tip, mostly between the meristematic region and the region of differentiation, occasionally in the region of elongation (Khera and Zuckerman, 1963). Mc Elroy and Van Gundy (1968) observed accumulation of protein and enlargement of nucleoli in cells adjacent and basipetal to the fed cells during the first six hrs feeding of H. arenaria on tomato. In next 6 - 12 hrs. the cytoplasmic contents were removed, and the nucleus distorted and/or reduced in size. The nucleolus was enlarged and the feed cells

appeared to double or triple in volume. The feed cells were later crushed and pushed to the root surface by the new meristem formed by the pericycle.

Most feeding by H. similis on cranberry was at the root tip with the stylet penetrating to the meristematic zone. However, a few nematodes fed further back from the tip and, where tissue differentiation had occurred, the stylet penetrated the xylem which were differentiated by the safranin staining of the lignified cell walls (Kiesiel, M. et al., 1971).

3.1.7. Criconemoides Taylor, 1936.

Ring nematodes' Criconemoides, feeding caused extensive lesions and pits on roots. Nematodes were observed with their anterior ends embedded several cell layers deep (Hung and Jenkins, 1969; Ratanaworabhan and Smart, 1970). C. curvatum and C. xenoplax were observed feeding near root tips. Single worms had either their stylet or part of their anterior end embedded in the root tissue. In cranberry root system, some root tips became discolored and ceased growing. In many cases a brown "frass like" material was seen near these discolored areas. Similar materials were not seen in areas where nematodes had not been feeding, suggesting that it was a result of nematode parasitism (Bird and Jenkins, 1964).

3.1.8. Hoplolaimus Dayday, 1905.

The "lance nematode" Hoplolaimus feeds on outer root tissues of many plants sometimes as ectoparasite and sometimes as endoparasite. Krusberg and Sasser (1956) found that H. coronatus caused considerable damage in cortex of cotton roots and formed cavities. Many undamaged cells were devoid of cell contents. Cell walls of damaged cells and deformed cortical cells appeared thicker and stained deeper with fast green than did undamaged cells. It penetrated the endodermis to feed easily on the vascular tissue. H. coronatus fed on phloem parenchyma and phloem elements, causing a death of these cells. The xylem elements those in close proximity of parasitized phloem were damaged even though no xylem elements had been attacked. Feeding and migration of H. coronatus caused extensive cortical damage in pine roots (Ruehle, 1962). Singh and Misra (1976) while studying sugarcane infected with H. indicus found that it caused damage to vascular parenchyma especially the endodermis and parenchyma after 10 days of inoculation. After 20 days the cavity enlarged and vascular elements became distorted. The roots became flaccid with completely disintegrated cortex and a few strands of xylem elements leading to wilting and yellowing of foliage. H. indicus remained confined to the cortex of sugar cane, cotton, soybean roots. It rarely crossed the endodermis. Much of the cytoplasm of invaded cells and some adjoining cells, disappeared or shrank together with the nucleus against the cell

wall (Singh and Misra, 1976; Lewis et al., 1976). Kourd and Osman (1980) observed that H. aegypti and H. columbus caused extensive damage to the cortical parenchyma and precipitation of tyloses in the stele region of soybean roots. Ng and Chen (1985) while studying histopathology of alfalfa roots infected by H. galeatus found hypertrophy and early cell division of host cells in the pericycle soon after nematode feeding began. As the pericycle cells increased in size and number, the endodermis flattened and collapsed. Vascular damage included feeding cavities, lysed phloem tissue, and the association of electron-dense material with the xylem elements.

3.1.9. Helicotylenchus Steiner, 1945.

The 'spiral nematode' Helicotylenchus invades the cortex, both as ecto-and endoparasite but does not move about actively in the tissue. Blake (1966) when inoculated banana with H. multicinctus, observed the nematodes feeding directly on cortical parenchyma evacuating their contents partially within three days of inoculation. After 4 days the nematodes entered the cortex wholly sometimes upto a depth of 4 - 6 cells. In cells in the vicinity of a nematodes' head, the cytoplasm had contracted, some cell walls were distorted or ruptured and the nucleus had increased in size. The cells were often discolored and some necrotic. The walls of cells invaded by the nematode and 6 - 10 adjoining cells were thickened (Orbin, 1973). Around the point of penetration of

nematodes small brown lesions were observed. In case of severe infestation sloughing off of epidermal cells and part of the cortex was also observed. The walls of cells invaded by the nematode took deep saf-ranine st-ain indicating the presence of lignin (Rao and Swarup, 1975). The nematodes' stylet penetrated the cytoplasm of food cells each of which was surrounded by 4 or 5 cells with an enlarged cytoplasm (Jones, 1978).

3.1.10 Rotylenchus Filipjev, 1936

Goodey (1935) recorded cellular destruction due to the formation of cavities, particularly in the area adjacent to the periderm, that were usually filled with nematodes, Rotylenchus bradys and their eggs in yams. He found neither hyperplasia nor hypertrophy. Golden (1956) found lesions formed by R. buxophilus extended into the cortex to varying depths. The lesions contained cellular debris, particularly broken cell walls and the cells adjacent to the lesions were devoid of protoplasm. Such damage was due to mechanical destruction of cells as a result of nematode movement, chemical attraction of cellular contents, and the removal of cell contents during feeding. Vovlas et. al., (1980) reported R. Laurentinus feeding semi-endoparasitically on small lateral carrot roots. Nematodes were partially embedded in the roots. The anterior ends of nematodes were penetrated in the wall of epidermal cells and some layers of the cortex. The vascular tissues were not damaged.

3.2. SEMI-ENDOPARASITES

The sedentary semi-endoparasites like Tylenchulus and Rotylenchulus, bring about many cytological, anatomical and physiological changes in the host.

3.2.1 Tylenchulus Cobb, 1913.

Citrus nematode, as it is commonly known as, enters the host cortex and makescavities in it. Van Gundy and Kirkpatrick (1964), inoculated Citrus sinensis and C. jambhiri with the larvae of T. semipenetrans, and found larvae singly or in groups in the epidermal and hypodermal cells. Young females penetrated cortical cells by rupturing the cell wall and then established at a depth of several cells in the cortical parenchyma. At the end of 4th week the females formed a permanent "feeding site" which consisted of 6 - 10 cortical cells "nurse cells", surrounding a cavity where nematode head was found. Cells surrounding the cavity were altered by nematode feeding as evidenced by dark staining reactions and cytoplasmic and nuclear changes. No increase in cell size or number of cells was observed due to nematode feeding. At later stages nematode feeding caused swelling of the cytoplasm. This proceeded until the cell was completely filled. There was an enlargement of the nucleus and nucleolus and a movement of nucleus to the central area of the cell. Advanced stages of cell change

were a thickened cell wall, very dense cytoplasm, and an amoeboid - shaped nucleus. There was no apparent effect on cells that impinged on the "nurse cell."

Schneider and Baines (1964) found that young females of T. semipenetrans penetrated midway or deeper into the cortex of C. aurantium and C. sinensis by breaking into one cortical cell after another and then became sedentary. The cells fed upon by the female nematodes did not die, but became greatly altered. The cytoplasm thickened and filled the cells, and the nucleus and nucleolus enlarged greatly. After successful host parasite relation ship failed to become established, the cortical tissue surrounding the nematode died, and the host isolated the area by wound responses. Cohn (1965) observed different stages of tissue deterioration in the feeding zone around the head of embedded female of T. semipenetrans in the cortical parenchyma of sweet lime, C. aurantifolia. Both the nucleus and nucleolus of host cells were enlarged, but the cell itself did not appear to increase in size.

3.2.2. Rotylenchulus Linford & Oliviera, 1945

Reniform nematode, Rotylenchulus reniformis chiefly a phloem feeder causes vast damage to the crops. While studying host parasite-relationship of Rotylenchulus reniformis on Gossyp-ium hirsutum, Birchfield (1962) observed that young females initiated

infection by destroying epidermal cells and causing a slight browning and necrosis of the surrounding cortical cells as they collapsed. Phloem cells near the head of the nematode stained darker than normal tissues; this apparent damage to the phloem extended several cells along the root axis. Necrosis in the phloem and parenchyma apparently resulted in severe root prunning of seedling roots and consequent dwarfing of cotton. R. reniformis on G. barbadense caused destruction of the epidermis and collapse of first 2 or 3 layers of the cortical parenchyma. The phloem cells became compact. Cells of the pericycle exhibited hypertrophy, along the axis of the root, and developed into giant cells (Oteiffa and Salem, 1972). Sivakumar and Seshadri (1972) found R. reniformis to be a phloem feeder in castor, papaya and tomato. The infected tissue at the feeding site showed hypertrophy, hyperplasia, thickening of cell walls, granular cytoplasm and enlarged nuclei. No changes were observed in the xylem. Cohn (1973) observed R. reniformis feeding in the stelar region of cotton, tomato and mint. The cells of the pericycle were hypertrophied and cell walls in endodermis were thickened. Some of the hypertrophied pericycle cells were multinucleate in feeding zone of R. reniformis in mint and there was break-down of cell walls in this zone in mint as well as upland cotton (Cohn, 1976). R. macrodoratus in oak and soybean induced a single, large and well defined

syncytium, extending deep into the stele, and bordered by a thickened wall which clearly isolated it from normal tissue (Cohn, 1976). R. reniformis in sweet potato roots fed in endodermis. The giant cells were observed in xylem parenchyma opposite to the feeding site. Isodiametrical cells were associated with the giant cells. (Brathwaite et al., 1974). In resistant and susceptible soybean roots, Rebois et al. (1974, 1975) reported some structural changes induced by R. reniformis. The female nematode penetrated intracellularly through the cortex to the endodermis where they inserted their stylets, secreted, and initiated syncytial formation and cell hypertrophy. Syncytia primarily involved pericycle tissues and to a lesser extent, xylem parenchyma and endodermis. Theyobserved initial cell of syncytium (ICS) usually in endodermis. Susceptible tissues exhibited two basic developmental phases the period of infection : an initial partial cell wall lysis and separation phase; and an anabolic phase, charactarized by organized proliferation and development accompanied by thick secondary wall deposits, which provided nutrition for sessile female development. The resistant or hypersensitive reaction (HR) lacked the anabolic phase and was characterized by a continued, , usually accelerated, and uncontrolled lysis phase. R. reniformis feeding, in cantaloup, stimulated the pericycle to either side of the endodermal feeding cell and caused hypertrophy with enlargement of the nucleoli and granular thickening of the cytoplasm (Heald, 1975)

The feeding area of R. reniformis on cowpea roots comprised of 6 - 12 cells on either side of the nematode head was observed by Razak et al. (1976). A group of 4 - 6 cells closest to nematode lips formed the "feeding zone". The "feeding cell" (or porosyncyte or initial cell of syncytium) was usually located near a proto-xylem pole. A "feeding peg" surrounded the nematode stylet where it penetrated the thickened cell wall of the initial cell. From the feeding pegs opposite the stylet aperture, emerged a coiled "feeding tube" (Razak and Evans, 1976). Robinson and Orr (1980) found that the feeding cell was of pericycle origin at two feeding sites, cortical origin at three feeding sites and of endodermal origin at 23 feeding sites in sunflower infected with R. reniformis.

3.3. ENDOPARASITES

The endoparasites completing their entire life cycle almost inside the host tissues, bring about most comprehensive anatomical, cytological and physiological changes inside the host causing great damages to plants. The endoparasites are of three types, migratory endoparasites comprising Pratylenchus, Radopholus and Hirschmaniella; parasites of aerial organs like Ditylenchus, Anguina, Aphelenchoides, Rhadinaphelenchus and Bursaphelenchus; and sedentary endoparasites containing Heterodera, Globodera, Meloidogyne, Nacobbus, Meloidodera.

3.3.1. MIGRATORY ENDOPARASITES

Migratory endoparasitic nematodes enter the plant tissues and move about actively. They feed on a cell, kill it, and move to an adjacent cell. The damages are in the form of necrotic areas, lesions and even tunnels.

3.3.1.1. Pratylenchus Filipjev, 1936.

Pratylenchus spp. are most commonly referred to as 'lesion' or 'root lesion' nematodes because of the conspicuous necrotic spots which they produce on the roots of infected plants. Pitcher et al., (1960) studied the effects of P. penetrans on apple seedlings under asceptic conditions. There was a rapid discoloration of the outermost (i.e. epidermis and hypodermis) and innermost (i.e. inner cortex and endodermis) cortical tissues, but little or no reaction in the intervening cortical parenchyma. In peach roots, all the cortical tissues were readily discolored. Histochemical test suggested that sensitivity to P. penetrans is correlated with the presence and concentration of phenolic substances in the various tissues of these two hosts. Pepper roots parasitized by P. penetrans were damaged due to the destruction of parenchyma cells of the root cortex (Shafiee and Jenkins 1963). P. coffeae fed primarily in the parenchyma of root cortex of

of Citrus jambhiri, when invaded a root tip, it often destroyed meristme and lateral root initiation usually occured near the destroyed root tip (Radewald, 1971). Oyekan et al., (1972) observed that within 24 hr. of inoculation of pea roots by P. penetrans the nematodes were mostly in the mid cortex which showed orange discoloration; their feeding and reproductive activities resulted in extensive breakdown of the cortex. No nematodes were observed within the stele. Corbett (1972) observed that P. fallax after penetration in root tips resulted rapid necrosis in the region of root hair development and the junction of main and lateral roots of wheat, barley and sugarbeet.

The axenic penetration and colonization of turnip (Brassica rapa) and corn (Zea mays) root tissues by P. penetrans produced brownish necrotic lesions. The nematode fed on both hosts, ectoparasitically for a while, on all zones of root tip except the root cap. After penetration it migrated around the Stelar regions in the cortex to produce large cavities. Although the penetration appeared to be mechanical, there was often a discoloration of the cells as the nematode advanced, suggestive for an enzymatic action (Ogiga and Ester, 1975). P. scribneri infection showed little cellular necrosis in snap bean, whereas cells surrounding the nematode in lima bean were extensively necrotic (Thomason et al., 1976). Olowe and Corbett (1976) found both

P. brachyurus and P. zeae in all parts of the maize roots including the stele. Cavities were found in the cortex with little accompanying necrosis and in the stele with much, including the deposition of a dense staining substance that occluded xylem and phloem tissues. P. zeae caused more mechanical damage but less necrosis than P. brachyurus.

Huang and Chiang (1976) studied the effects of P. coffeae on sunki orange (Citrus sunki) in the green-house and found nematodes feeding primarily in the cortex, both inter-and intracellularly, resulting in extensive cellular destruction. The nematode did not penetrate the endodermis and did not cause hyperplasia, hypertrophy or any stimulation in cellular growth at the infection sites. Cells at infection sites showed retraction and disappearance of cytoplasm, thickening of cell walls and necrosis of cells around feeding sites (Inserra et al., 1980; Acosta and Malek, 1981; Onapitan and Amosu, 1982).

3.3.1.2 Radopholus Thorne, 1949.

Burrowing nematodes, Radopholus spp. cause injury essentially like that attributed to Pratylenchus spp. Females and larvae all enter the root and destroy cortical cells, thus producing small necrotic lesions which enlarge with invasion by bacteria and fungi. Du Charme (1959) found that R. similis entered actively

growing root tips in the region of cell elongation and root hair production in citrus roots. As the nematode progressed through the root, tunnels and cavities were formed in the cortex and stele. All types of parenchyma tissues were attacked. Inside the root the nematodes entered the stele through the passage cells of the endodermis. The phloem cambium ring, where accumulated in large numbers, seemed preferred. Cells exposed to the nematode metabolites sometimes became hypertrophied. Hyperplasia and tumour formation from the pericycle occurred when the nematodes passed through the endodermis and became situated next to the pericycle. These pericycle tumours were also attacked by the nematodes. Blake (1961) found R. similis in lesions in the root cortex of banana, containing females, larvae and eggs, originated as a puncture in the epidermis of the root. Beneath the epidermis was a zone of necrotic cells. The cells forming the boundary of the lesion showed mild hypertrophy. Within 12 hr of entering the cortex, the size of the nucleus and nucleolus in the few cells immediately surrounding the nematode had increased significantly. This suggested that the nematodes fed directly on the cytoplasm of the cell (Blake, 1961, 1966). O' Bannon et al., (1967) made histological comparisons in four citrus rootstocks infected with R. similis. In all four varieties; i.e. 'Ridge pineapple' and 'Milam' resistant; 'Estes' tolerant and 'Duncan' highly susceptible to R. similis, the

nematodes penetrated the epidermis, cortex and endodermis, causing cell wall disintegration, hypertrophy, hyperplasia, and wound gum formation. Entire vascular cylinder was invaded in 'Duncan' and 'Milam' but not in 'Ridge pineapple' and 'Estes'. There was little or no nuclear change in 'Estes' and 'Duncan'. However, in 'Milam' and 'Ridge pineapple roots the nuclei and nucleoli in cells adjacent to the infected area became enlarged. R. similis penetrated the tissues in rhizomes of ginger (Zingiber officinale) intracellularly forming channels or galleries (Vilsoni et al., 1976).

3.3.1.3. Hirschmaniella Luc & Goodey, 1963.

Hirschmaniella gracilis causes a disease of rice called 'Mentek' beginning as root rot with small discolored areas which gradually enlarge involving the entire cortex. In severely infected plants only the epidermis and central cylinder remains, the parenchyma being totally destroyed. Secondary roots and root hairs become dead (Jenkins and Taylor, 1967).

Babatola and Bridge (1980) studied the feeding behaviour and histopathology of H. oryzae, H. imamuri and H. spinicaudata on rice. They found lesions extending along the burrowed channels of the nematode for some distance from the entry point, until, eventually the nematodes were surrounded by broken non-necrotic

cells. Mechanical damage caused large cavities and collapse of cell walls, mainly within the cortex. The stele was damaged only in roots infected by H. spinicaudata. The burrowing activities of H. imamuri almost severed primordia of lateral roots. Cortical and lacunal cells were collapsed, and cell walls were broken by nematode feeding.

3.3.2. SEDENTARY ENDOPARASITES

Nematodes of several genera are adopted to a sessile life within host plants. Adult swollen females remain in one position and induce formation of specialized cells in the host. These cells survive repeated removal of portions of their contents.

3.3.2.1. Heterodera Schmidt, 1871.

The cyst forming nematode Heterodera is primarily a root parasite though it may also occur on underground stems, e.g., stolons and tubers of potato (Oostenbrink, 1950) and, exceptionally, on leaves of white clover Ross, (1960). Syncytial formation appears to be an essential phenomenon of successful parasitism in Heterodera. Syncytia induced by cyst-nematodes originate in the pericycle, endodermis, or adjacent cortex, frequently opposite protoxylem poles (Endo, 1978). H. oryzicola infestation on rice

var. CRM - 13 - 3241 roots caused cell wall disintegration of a group of phloem parenchyme cells of stele and formed a syncytia near the head region were highly thickened and the degree of thickness gradually declined in cells away from the nematode. The cyst nematode broken open the cortex and epidermis to protrude outside the root (Jayaprakash and Rao, 1982).

Penetration :

Mankau and Linford (1960) observed that H. trifolii penetrated clover roots within 14 min after coming into contact with the root surface. The larvae generally entered the young proliferous zone of the roots, but penetration was also common along the length of the root upto the mature tissue.

Formation of Syncytium :

The juvenile inserts its stylet into the initial cell of syncytium (ICS), and around this a plug develops (Endo, 1978). The wall near the stylet does not dissolve, but becomes evenly thickened as the syncytium develops. Walls internal to the stylet are degraded, however, so that protoplasts of neighbouring cells fuse. Wall dissolution occurs at pit fields, where the wall is thinnest. Affected cells also expand, enlarging wall gaps. Syncytia initiated in the cortex extend towards the stele, with relatively little cell expansion. Cells incorporated within

the stele expand considerably, especially pericycle, vascular parenchyma, and cambial cells. Within the stele, extension of the syncytium occurs longitudinally on both sides of the initial region to reach a length of upto 2 - 3 mm. Cell wall dissolution, in Heterodera infection, in the process of syncytial formation has been reported on a large number of plant species (Nemec, 1932; Mankau and Linford, 1960; Endo, 1964, 1965; Ambrogioni and Porcinai, 1972). Syncytial walls of Heterodera infection were especially thickened near the lip region of the nematode and along the syncytial walls adjacent to the xylem (Triffit, 1931; Nemec, 1932; Mankau and Linford, 1960; Endo, 1964). Cell wall thickening was minimal where syncytia appear to merge with normal tissues. In an electron microscopic study of the early stages of H. glycine infection of soybean, Gipson et al., (1969) stated that cell wall disintegration appear to be the initial phenomenon leading to syncytial development. Within 42 hr of inoculation, abnormal perforations of cell walls were visible. Progressive deterioration of cell walls occurred such that by 196 hr after inoculation portion of the cell walls were no longer present.

Nuclei and Nucleoli in Syncytia :

Syncytia induced by nematodes have been reported to attain the polynuclear state by (1) mitoses in the absence of cytokinesis, (2) amitosis or nuclear fragmentation, and (3) the merging of

nuclei from several cells that coalesce as a result of cell wall dissolution. Within the polynuclear syncytium, nuclear hypertrophy has been attributed to swelling, nuclear coalescence, and fusion or incorporation of chromosomal sets. Tischler (1902) reported that an increase in the number of nuclei in young giant cells initially occurs through normal mitosis. Later, further multinucleation involved amitosis or nuclear fragmentation through budding, in which nuclei gave rise to a whole series of new nuclei. In a report of giant cells in galled glorybower (Clerodendron) roots, Nemec (1910) observed that when the head region of the nematode reached the plerome, the host cells surrounding the oral opening of the larvae enlarged. The plasma content of these cells increased, and their nuclei divided without the formation of cell walls; thus, multinucleation of cells occurred. Owens and Specht (1964) found that multinucleation in the early stages of syncytial development was due to the dissolution of host cell walls and the coalescence of their protoplasm. In the study of H. glycines infection of soybean root (Endo, 1964), mitosis was not evident in syncytia. Nuclear morphology of syncytia of clover roots infected by H. trifolii was described by Mankau and Linford (1960). Upon stimulation by the nematode, the cell nucleus enlarged and the cytoplasm increased in density and became hyperchromatic. Cells adjacent to syncytia showed nuclear enlargement and cellular hypertrophy, which was followed by a dissolution of

the cell wall and a merging of cytoplasm.

Syncytial Cytoplasm :

The granular texture of cytoplasm of giant cells in Heterodera spp. infections has been reported for numerous host-parasite interactions. Bergman (1958) noted the fine granular texture of syncytial cytoplasm in sugar beets during early stages of H. schachtii infection. At later stages, the cytoplasm became turbid and stained heavily with fuchsin. The persistance and texture of syncytial cytoplasm in soybean roots infected by H. glycines varied with the feeding habits of the male and female nematode (Endo, 1964). Initially both sexes stimulated a reticulate cytoplasmic network which later became granular. Syncytia induced by males were ephemeral, and their deterioration began within a few days after inoculation. This is indicative of the relatively short feeding time of developing male larvae. The extensive syncytial development and dense granular cytoplasm, characteristic of syncytia induced by female nematodes, can be attributed to the prolonged feeding time required for their growth and development. Gipson et al. (1969) reported on the ultrastructure of syncytia induced by H. glycines. They found that in the first 6 days of development, the central vacuoles of the

cells being incorporated into the syncytium appeared to be replaced with cytoplasm containing large numbers of microtubules and resembling smooth endoplasmic reticulum. The remaining vacuoles progressively decreased in size as the syncytium developed. Mankan and Linford (1960) reported the presence of hyaline, tubular structures in the syncytial cytoplasm of host cells stimulated by H. trifolii. Similar elongated bodies were observed (Endo, 1964) in syncytia near the larval lip region of the soybean cyst nematodes feeding on soybean roots.

3.3.2.2. Globodera Skarbilovich, 1959.

Potato cyst nematode (potato root eelworm, golden nematode, formerly Heterodera rostochiensis) Globoderra rostochiensis larvae after entering a potato rootlet usually settle to feed with their head end in the vascular tissue. Certain cells in the stele around the head of the nematode enlarge and coalesce to form syncytia, which is essential for normal female development (Webster, 1972).

Feldmesser (1952, 1953) reported for the first time root galling on tomato roots infected by G. rostochiensis. Large syncytia (giant cells) were developed during third developmental stage of the nematodes, in the cortex and in the stele causing discontinuity of some vascular elements and the retardation of

other vascular elements. During the fourth and fifth stages giant cell areas became more extensive, and individual giant cells became larger and more irregular in shape, cutting off greater areas of vascular tissue. The processes involved in the formation of giant cells are fragmentation and dissolution of cell walls, coalescence of the cytoplasmic masses of the previously intact cells the aggregation of the nuclei from these cells.

In potato roots infected with G. rostochiensis, giant cells were confined to pericycle and parenchyma cells of the vascular strand between the primary xylem and phloem groups. Giant cell formation was much less frequent in the cortex (Cole Howard, 1958). Hyperplastic reaction in the cells of tomato roots infected with G. rostochiensis were differentiated into three types by Sembdner (1963) : (1) Extensive irregular cell division proceeding mainly from the central cylinder. (2) Multiple subdivision of single cells in the endodermis, in the neighbouring layer of cortex parenchyma and/or in the endodermis. (3) Hyperplastic reactions in a wound-periderm-like manner. Kaczmarek and Giebel (1980) reported mitotic disturbances in presence of G. rostochiensis in the cells of lentil (Lens culinaris). IAA and CHA (chloragenic acid) stimulated most miotic disturbances, e.g., mitosis inhibition, various chromosomal aberrations, lack of cytokinesis, and the formation of 2 - or 3 - nucleate cells.

3.3.2.3. Meloidogyne Goeldi, 1887.

Infection with root knot nematodes (Meloidogyne spp.) stimulates the formation of a variable number of discrete giant cells (usually about 6, but reported to vary from 2 to 12) in host tissues. Hyperplasia and hypertrophy often surrounded the region of infection and usually caused galls to be formed terminally or sub-terminally on the infected roots.

Penetration :

Root-knot nematode larvae readily penetrate plant roots near the apical meristem (Nemec, 1910), within 24 hrs after inoculation (Siddiqui & Taylor 1970); however, other regions of the root are not immune to attack (Christie, 1936). Once in the root, migration occurs both inter- and intracellularly (Nemec, 1910 ; Christie, 1936; Krusberg and Nielson, 1958).

Formation of Syncytium :

The formation of giant cells or syncytia through the dissolution of cell walls and the coalescing of their contents was reported for root-knot infection of Nicotiana hybrids (Kostoff and Kendall, 1930). Christie (1936) gave further support to this process of syncytial formation in the study of root-knot infections of tomato. He observed that undifferentiated cells near the head

of the nematode enlarged slightly and, following a slight swelling of nuclei, the cell walls adjacent to the initially stimulated cell disintegrated. There was progressive dissolution of cell walls followed by the coalescence of protoplasm during the expansion of the giant cell. Similar evidence of cell wall dissolution has been reported for a number of hosts infected by species of Meloidogyne (Krusberg and Nielsen, 1958; Dropkin and Nelsen, 1960; Owens and Specht, 1964; Littrell, 1966). In contrast, Huang and Maggenti 1969; Paulson and Webster, 1970; Jones and Northcote, 1972; Jones and Dropkin, 1976; Jones and Payne, 1978 found no evidence of cell wall dissolution or breakdown in the formation of syncytia in root-knot infections of Vicia faba when syncytial boundaries were observed through the electron microscope. They concluded that cell wall breakdown played no part in giant cell formation and the multinucleate condition arose primarily from mitosis without cytokinesis.

Nuclei and Nucleoli in Syncytia :

The multinuclear state in syncytia induced by root-knot nematodes have been reported to arise as described in case of Heterodera infections (Tischler, 1902; Nemec, 1910; Krusberg and Nielsen, 1958; Dropkin and Nelson, 1960; Owens and Specht, 1964; Littrell, 1966). Owens and Specht (1964) found that number of nuclei within the developing syncytium could be correlated with

the number of host cells that would normally occupy the volume of the syncytium. Thus multinucleation in the early stages of syncytial development was thought to result solely from the dissolution of host walls and the coalescence of their protoplasm. Nuclear changes ranged from a nucleus near the stylet of the nematode which showed a hypertrophied nucleolus with the apparent absence of a nuclear membrane, to nuclei with various stages of membrane deterioration and a lobulated periphery. Other nuclei aberrations included nucleoler fragmentation so that small granules, which stained like nucleoli, were scattered throughout the nucleus. Irregularly shaped dumbbell,-or sickle - shaped nuclei were observed. Similar observations of sickle shaped nuclei were reported on root-knot infections of sweet potato (Krusberg and Nielsen, 1958). Nuclear enlargement in syncytia of root-knot infected tomato, cucumber, and hawk's - beard (Crepis capillaris) (Owens and Specht, 1964) appeared to result from swelling and in some cases from nuclear fusion. Such nuclear fusion in syncytia of tomato account for the extreme enlargement of some nuclei which had diameters of 35μ as compared to normal cell nuclei of 6 μ. Other data (Rubinstein and Owens, 1964) indicate that a 10 - 12 - fold increase in nuclear volume is more usual in tomato roots. Hairy vetch plants inoculated with M. incognita had syncytial nuclei with over 100 chromosomes, whereas normal cells have a 2N number of 14 (Dropkin 1965).

Syncytial Cytoplasm :

The granular texture of syncytial cytoplasm of giant cells in root-knot infection has been reported for a number of host-parasite interaction (Christie, 1936; Owens and Specht, 1964; Heald, 1969; Riffle, 1973). There was a tendency for the syncytial cytoplasm near the head region of the nematode to be more dense and hyper-chromatic than the remainder of the syncytial contents (Christie, 1936). As the nematode matures and nutrient demands from giant cells increase with egg laying, the giant cell cytoplasm shows signs of intense metabolic activity. Nuclei become highly lobed and heterochromatic with prominent and numerous nuclear pores, indicating rapid nucleus - cytoplasm exchange. Starch grains are lost, and the secondary vacuoles become more numerous and smaller. Finally, the cytoplasm becomes extracted as the giant cells senesce, leaving some organelles and the ingrowths, but little ground cytoplasm.

3.3.2.4. Gall Formation :

Mani (1964) describes plant galls as "pathologically developed cells, tissues or organs of plants that have arisen mostly by hypertrophy (over-growth) and hyperplasia (cell proliferation) under the influence of parasitic organisms). Galling is the one of the earliest host responses of root-knot nematode

in the roots of the plants. Schuster and Sullivan (1960) reported that galls were induced in tomato roots by M. incognita larvae even when they did not enter the tomato roots, thus concluding that the stylet penetrated the root surface and secreted materials that stimulated host tissues to form galls. Davis and Jenkins (1960), reported gall formation in Gardenia sp. infected with M. incognita, M. incognita acrita and M. hapla. Cortical and stelar proliferation accompanied all infections. In plant infected with M. hapla although cortical and stelar parenchyma proliferated extensively, cortical cells exhibited little hypertrophy. This lack of increase of cell size probably accounts for the smaller gall size characteristic of M. hapla infected roots. Eversmeyer and Dickerson (1966) observed two types of galls on peony roots infected with M. incognita and M. hapla . 'Regular' type of swellings involved the entire surface of the root, whereas the 'side' type involved only a limited portion. Huang (1966) reported gall formation on the fibrous and fleshy roots of ginger (Zingiber officinale). Abnormal xylem and hyperplastic parenchyma were also present several small abnormal galls on newly formed adventitious roots as well as on rootlets of tap root of Solanum melongena infected with M. incognita acrita were found by Varghese and Kamlesh (1970). Gall formation by M. naasi in wheat and oat roots was studied by Siddiqui and Taylor (1970); Siddiqui (1971a, 1971b,). Slight root galling and

thickening of roots was found in conifers Thuja orientalis and Juniperus horizontalis infected with M. incognita. The galling in conifers was deviated from typical root gall-ing in other plants (Nemec and Morrison, 1972). Gall formation in different plants infected with M. incognita has been reported by Talat Farooq (1973); Ibrahim et al., (1973); Mc Clure et al., (1974); Ngundo and Taylor (1975); Jones and Payne (1978); Arens et al., (1981); Glazer et al., (1983).

Mechanism of Gall Formation :

Beille, as early as 1898, suggested that giant cell formation involved cell wall dissolution and subsequent cytoplasmic fusion between the neighbouring cells; this view was supported by Christie (1936); Krusberg (1963); Birchfield (1965); Bird (1973, 1974); Rohde and Mc Clure (1975). Nemec in 1910 gave an alternative explanation for the mechanism of formation of giant cells. He observed that nuclei in giant cells induced by a root knot nematode divided without forming new cell walls. The same was observed by Krusberg and Nielsen (1959); Bird (1961); Owens and Specht (1964); Smith and Mai (1965); Littrell (1966). Swamy and Krishnamurthy (1971) and Prakaso Rao et al., (1973) reported that the gall formation was attributed to the hypertrophy and hyperplasia of parenchyma cells and partly of vascular cells in M. javanica infected plants. Ibrahim and Massoud (1974) reported that M. javanica was found feeding in cortex, endodermis, pericycle

and stele of soybean roots resulting hypertrophy, hyperplasia and giant cell formation in the tissues immediately surrounding the nematode head and consequently developing galls.

Galls on Above-ground Plant Parts :

Sometimes root-knot nematodes cause galls on above-ground plant parts. Linford (1941) inoculated stems, petioles and leaves of tomato and cowpea and obtained galls. M. incognita caused leaf galls in Siderasis fuscata (Miller and Di Edwardo, 1962). Wong et al., (1969) observed gall formation in aerial parts of tomato inoculated with M. javanica. Slight swellings appeared around the point of infaction a few days after inoculation due to hypertrophy and hyperplasia of the cells around the nematode head. Gall formation on in aerial parts of plants like stem, leaf, petiole, inflorescence was also observed by Steiner (1940); Fassuliotis and Deakin (1973); Taylor (1976); Mac Gowan et al., (1979); Lehman and Mac Gowan (1983); Lehman (1985); Lehman and Mac Gowan (1986).

Induction of Gall Formation :

Root-knot nematode, once within the root, secretes a material which causes hypertrophy and to some extent hyperplasia. The comprehensive study of root-knot nematodes on tomato roots by Christie (1936) and the study of galling responses reported

by the early workers Treub (1887) and Nemec (1910, 1911), have laid foundation for recent studies on the agents of gall formation. The process of gall ing by nematodes which is brought about largely by hyperplasia and syncytial formation, appear to be two quite different responses. The former starts relatively rapidly, i.e., within a few hours of infection, and may be larval feeding at the root surface without actually entering the root (Lo wenberg et al., 1960; Schuster and Sullivan, 1960). The different shapes and structures of the syncytia induced by the four Meloidogyne species in tobacco provide some insight into the origin and development of syncytia. The compact rounded syncytia associated with M. incognita and M. hapla appear to be the result of increased nuclear activity without subsequent cytokinesis. In contrast the formation of very extensive, highly lobed giant cells associated with M. arenaria and M. javanica on tobacco may involve cell dissolution and coalescence of cytoplasm (Sosa - Moss et al., 1983). The role of growth promoting substances are proposed to be involved in the production of galls by Ustinov (1951); Owens and Specht (1964); Yu and Viglierchio (1964); Dropkin (1972).

Muge (1956a, 1956b) suggested that amino acids which were in much greater concentration in galls than in healthy tissues were probably responsible for galling and that possibly liberation of amino acids by nematodes caused the growth of the gall. Goodey

(1948), for the first time suggested that galls were initiated by excretion, similar to auxins. Auxins have been identified in galls caused by root-knot nematodes, and frequently greater auxin concentrations occur in nematode infected tissue than in non-galled tissue (Balasubramanian and Rangaswamy, 1962; Setty and Wheeler, 1968). Certain auxins are unique to galled tissue and their concentrations are influenced by the Meloidogyne and host species involved (Yu and Viglierchio, 1964; Viglierchio and Yu, 1968). Cytokinin levels may also increase in Meloidogyne-infected plants (Krupasagar and Barker, 1966), and have been reported to be higher in noninfected root-knot susceptible plants than in resistant plants (Van Staden and Dimalla 1977). Auxins alone (Kochba and Samish, 1971), cytokinins alone (Dropkin et al., 1969), and combinations of these two (Kochba and Samish, 1971; Sawhney and Webster, 1975), when exogenously supplied to it root-knot resistant plants, reversed the resistance response and made plants susceptible to infection by Meloidogyne juveniles. Auxins and cytokinins have also been identified in different stages of Meloidogyne (Viglierchio and Yu, 1968; Dimalla and Van Staden, 1977). If growth regulators are involved in giant cell formation, yet it is not clear whether they are of nematode or host origin. Ethylene, another plant growth regulator, has been associated with gall formation. Ethylene production was highly correlated with increases in gall weight on M. javanica infected, excised tomato

roots, suggesting that it may be involved in the hypertrophy of cortical parenchyma tissue during gall formation (Glazer et al., 1985).

Effect of Galls on Vascular Elements :

The host-parasite relationship of the root-knot nematode infection causes disruption of xylem and phloem tissues resulting in hindrance in the transportation of water and mineral nutrients and translocation of food materials in the host plan. Davis and Jenkins (1960) observed the disruption of stele in Gardenia sp. infected with Meloidogyne sp. by giant cells. Proliferated parenchyma cells caused the conducting tissues to scatter so that xylem and phloem occurred in irregular patches rather than in one column. They also observed a periderm like formation developing from the outer layers of cortex on the infected site of the root, even though the nematode was embedded several cells beneath the surface. In ginger (Zingiber officinale) infected with M. incognita, wound cork and lignified wall thickenings of endodermis and pericycle are reported at infection sites (Huang, 1966). Odihirin and Jenkins (1965) also reported disruption of vascular cylinder so that the continuity of xylem and phloem is broken. Talat Farooq (1973) found the division of stele in root galls of Licopersicon pimpinellifolium infected with M. incognita. When the giant cell complex was in the vascular cylinder, the abnormal

vessels connected with the phloem fibres; when the giant cell complex was in the cortex, vessels were independent of surrounding cells (Ediz and Dickerson, 1976). In infected roots the anterior ends of the larvae were invariably embedded in the phloem and the giant cells were frequently bordered by xylem vessels. This resulted in a morphological aberration of the vascular cylinder (Finley, 1981). The formation of abnormal xylem has been reported by different workers in different plants infected with root-knot nematodes (Litterel, 1966; Siddiqui and Taylor, 1970; Siddiqui et al., 1974; Ngundo and Taylor, 1975; Jones et al., 1976; Byrne et al., 1977; Meon et al., 1978; Jones 1981; Pasha et. al., 1987). Eversmeyer (1966) reported vascular tissues in Peony roots in which xylem and phloem were mostly parenchymatous having much starch. Riffle (1973) observed that xylem trachieds in immediate vicinity of giant cells were distorted, crushed and even scattered in irregular isolated patches. The complete suppression of xylem and phloem tissues has also been reported (Wong and Willets 1969; Swamy and Krishnamurthy, 1971). Siddiqui and Ghouse (1975) found that after the destruction of primary phloem at the site of infection abnormal elements with unusual orientations are formed in the roots of Lagenaria lencantha infected with M. incognita. Pasha et al., (1987) found that abnormal xylem occurred in irregular patches with scattered vessel elements resulted in discontinuity of vascular tissues.

Effect of Galls on Physiology of Plants :

The root-knot nematode not only induces cytological and anatomical changes in the host plant but they also affect the host physiology in one way or the other.

i) Respiration :

Muge (1956) found that respiration of Meloidogyne induced galls of cucumber was three times greater than that of non-infected root tissue. Bird (1962) found that respiration of gall tissue was no greater than that in adjacent tissues or in un-infected roots in tomato. The effect of Meloidogyne infection on respiration rate varies with nematode-host combinations and plant age, increased enzyme activity (Veech and Endo, 1969) and changes in biochemical components of giant cells Owens and Specht (1966) show that giant cell metabolism is affected and regulated by the nematode.

ii) Translocation of Water and Nutrients :

Histological studies (Byrne et al., 1977; Meon et al., 1978) of Meloidogyne galls revealed a disruption in the continuity of vessel elements and the presence of abnormal vessel elements. Such alteration disrupt nutrient and water flow in the plant. Hunter (1958) studied nutrient absorption in tomato infected by M. incognita. He found suppressed shoot growth and chlorotic

foliage on diseased plants with no accompanying alteration of N, P, K, Ca, Mg and Fe content in the foliage. Roots of infected plants, however, contained larger quantities of N, P, K and Mg than did uninfected plants. Translocation of other substances is impaired in Meloidogyne-infected plants. Nicotine, which is synthesized in tobacco root tips, increased in root tissue of susceptible and resistent plants and in leaves of only resistant plants following M. incognita infection (Hanowanik and Osborne, 1975). However, leaf content of nicotine decreased by half in the susceptible cultivar where the root damage was the greatest. This occurrence indicates that translocation may have been affected.

O' Bannon and Reynolds (1965) reported that cotton plants infected by M. incognita consumed slightly more water than noninfected plants when water was supplied to the plants continuously. Meon et al., (1978) found that water flow in infected tomato roots decreased with increased inoculum densities and with decreasing soil moisture content. They concluded that disruption and development of vascular elements resulted in restricted water flow.

iii) Photosynthesis :

Meloidogyne infection of roots decreases the rate of photosynthesis in leaves. Loveys and Bird (1973) reported that

high inoculum levels of M. javanica on tomato caused a decline in net photosynthetic rate within two days after inoculation. This lower rate was maintained throughout subsequent growth of the infected plant. Wallace (1974) reported that decrease in the photosynthetic rate of young tomato plants varied with inoculum levels and was not greatly influenced by plant age.

iv) Biochemical Constituents :

The protein content of tomato syncytia has been shown to be about 4 - fold in 22 days old syncytia (Bird, 1961) and also to range from 3-to 20-fold depending on age (Owens and Specht, 1966) and to about 3-fold in soybean and 10-fold in balsam (Gommers and Dropkin, 1977). Balasubramanian and Purushothaman (1972) studied the phenolic contents of root-knot affected tissues and observed not much difference in total phenolic contents in galled and healthy roots. However, orthodihydroxy phenolic content was slightly higher in galled tissue than in normal tissues. Veech and Endo (1969) reported increased activity of enzymes like alkaline phosphatase, acid phosphatase, peroxidase, adenosine triphosphatase, esterase and cytochrome oxidase at the site of infection than in adjacent non-infected tissue. Owens and Specht (1966) found that in galled tissues carbohydrates and pectins decreased 36% and cellulose and lignin decreased 31%. Other components in the gall increased as follows : hemicelluloses

36%, organic acids 67%, free amino acids 304%, proteins 80%, nucleotides 29%, RNA 87%, DNA 70% lipids 154%, and minerals 4%. Several workers Endo and Veech, (1969) Veech and Endo, (1969), (1970) Orion et al., (1973); Wang and Bergeson, (1974); Lewis and Mc Clure, (1975); Jones and Payne, (1978); Nasr, et al. (1980); Rashid, et al. (1981); Haseeb et al. (1982); Basu, et al. (1983), Ganguly and Dasgupta, (1983), have carried out many experiments to establish a relationship between an increase or a decrease of biochemical constituents and root-knot infection.

3.3.2.5. Nacobbus Thorne & Allen, 1944.

False root-knot nematode, Nacobbus causes galling through both hypertrophy and hyperplasia. The cells resemble Meloidogyne-induced giant cells occurring in the cortex adjacent to the stele. These cells, however, are described as spindle-shaped area of amorphous tissue with atypical cell walls which collapse and coalesce (Jenkins and Taylor 1967).

Clark (1967) observed damage to the host roots caused by N. Serendipiticus larval feeding, followed by necrosis of cortical cells. Galls were associated with adult females containing a spindle shaped mass of small cells having starch grains. Jones and Payne (1977) found a clear cut boundary between the gall cells surrounding the syncytium and the transformed cells in

tomato roots,infected with N. aberrans. Syncytia cells were interconnected by perforations through their walls.

According to Inserra et al., (1983), all the juveniles of N. aberrans invaded cortical parenchyma of sugarbeet roots and formed cavities in the cortical cells. Cells with dense cytoplasm and hypertrophic nuclei were seen in cortex and endodermic cells as a result of nematode feeding. In some cases cavities extended from the cortical parenchyma to the periphery of the central cylinder.

3.3.2.6. Meloidodera Chitwood, 1956.

Pine cystoid nematode, Meloidodera does not produce root galls. Giant cells adjacent to the head of the female of Meloidodera , developed in the region of cortex, in protoxylem and protophloem in both slash and loblolly pine roots. The part of the giant cell wall in contact with the head of the female nematode became thicker than the remaining wall area. Hyperplasia of the cortical and vascular parenchyma cells surrounding the giant cells was very common. Trachieds around giant cells were somewhat pushed out of position, but their continuity remained unbroken. The cells adjacent to the swollen female were collapsed and compressed (Ruehle, 1962 and Cohn et al., 1984).

Mundo et al., (1983) studied giant cell formation in loblolly pine, peony and sage infected with M. floridensis, M. charis and M. belli respectively. M. floridensis initiated the giant cell in the pericycle of pine, and it was restricted to the inner periphery of the vascular cylinder. The giant cell remained surrounded by vascular parenchyma cells, which were continuous with those of the pericycle. Hyperplasia commonly occurred in cells adjacent to the giant cells, however, no hypertrophy or necrosis was observed. M. charis and M. belli also initiated giant cells in the pericycle, but as the cells enlarged they extended into the vascular cylinder which in some cases became distorted. The mature giant cell directly contacted xylem and phloem which were major components of the vascular cylinder. Both hyperplasia and hypertrophy of the adjacent tissues occurred.

3.3.3. PARASITES OF AERIAL ORGANS

A few of the plant - parasitic nematodes are associated with the diseases of flowers, leaves and stems. They cause extensive damages in the form of lesions and tunnels in nonvascular tissues.

3.3.3.1 Ditylenchus Filipjev, 1936.

Swollen tissues associated with stem nematodes Ditylenchus spp. have the same general histological appearance regardless of

the host. Cells of the cortical parenchyma are enlarged, more spherical, and more numerous in the swollen areas, with large intercellular spaces. As early as 1927 Quanjer suggested that nematodes release a pectinase during feeding resulting in a dissolution of the middle lamella; this allowed cells to become more spherical and produced larger intercellular spaces. Potato tubers infected with D. dipsaci and D. destructor usually have a cracked epidermis with sunken lesions and a dry rot condition. Lesions caused by D. destructor increase in size as disease progresses, giving rise to the production of a reticulate system of cavities and tunnels. Cytoplasm and nuclei become oriented toward the side of the side closest to the nematodes. Cortical cavities are found within roots parasitized by D. destructor. In addition, hypertrophy and hyperplasia occur in both the stele and the cortex (Jenkins and Taylor, 1967).

Feder and Feldmesser (1953) pointed out that D. dipsaci infection of Narcissus pseudonarcissus resulted in "Spikkeled" areas on leaves, sickle-shaped leaves, stunted unsaleable flowers and eventual destruction of the bulbs. The external leaf morphology and the raising of the "spikkeled" area above the leaf surface were the results of hypertrophy and hyperplasia in areas adjacent to nematode loci. Groups of affected epidermal cells enlarged and buckled away from underlying tissue forming loculate

"spikkels" containing the nematodes. Giant cells were often found in areas containing D. dipsaci, and were characterized by heavily stained cytoplasm, smaller vacuoles and abnormally large, often pycnotic cells. Mitotic activity was not observed.

The effects of two populations of nematodes, Waynessville, North Carolina (WNC) and Raleigh, North Carolina (RNC) on Alaska pea and Wando pea were studied by Hussey and Krusberg (1968, 1970). WNC gave resistant response (necrosis) while (RNC) gave susceptible response (galling). In WNC - infected seedlings, seven days after inoculation, necrosis in the older parts was more extensive, although still restricted to the epidermis and adjacent tissue. After 14 days, necrotic cells were still mostly continued to the epidermis, but in some sections, adjacent cortical cells were also affected. These cortical cells had undergone mitotic divisions giving the appearance of cork formation. Cells in hyperplastic areas were much smaller than adjacent unaffected cortical cells, and the hyperplasia was directed toward the necrotic cells. In RNC-nematodes inoculation seedlings, tissue disruption, a susceptible response, was observed. Large cavities bordered by greatly misshapen cells occurred in the cortex and parenchymatous mesophyll tissue of infected seedlings 7-days after inoculation. The large cavities resulting from destruction of parenchymatous cells, extended acropetally into the embryonic

leaves and basipetally into the stem. Epidermis adjacent to infected tissue was hyperplastic and convoluted. No necrosis was observed in tissues galled by RNC nematodes. After 14-days of inoculation, the damage was more extensive causing general destruction of the cortex. Portions of the epidermis were destroyed. Outer layers of the cortex were necrotic and partially sloughed, with the adjacent cortical cells dividing and giving the appearance of cork formation.

Reed et al., (1979) inoculated Buffalo (susceptible) and Washoe (resistant) varieties with D. dipsaci. Sections of galls of Buffalo showed large cortical cavities with darkly stained and granular cells on the peripheries. Some hypertrophied cells surrounding cavities were evident. Nematodes were found in all parts of the cortex from just inside the epidermis to near the vascular cylinder. Similar galls developed in Washoe, but cellular destruction and cavity formation were less extensive than in Buffalo. Washoe had fewer enlarged cortical cells with granular cytoplasm than Buffalo. Stem galls on Citrus arvense by D. dipsaci were characterized by extensive hypertrophy and hyperplasia, differentiation of nutritive tissue, nuclear modification and a central cavity containing nematodes (Watson and Shorthouse, 1979).

3.3.3.2. Anguina Scopoli, 1777.

Seed and Leaf gall nematode, Anguina feeds ectoparasitically on young leaves near the growing point (Southey, 1982). Anguina tritici produces galls from undifferentiated flower buds. Staminate tissues are first involved than carpellate tissues. Finally tissues between carpels and stamens are involved. Larvae of fescue leaf gall nematodes (A. graminis) enter the tissues of young leaves and form galls resembling knots or swoller nodes.. All portions of leaf tissue become enlarged and hypertrophied, the cells being much larger than normal. A. graminophila form galls on the leaves of fine bent-grass. Transverse sections reveal that cell hypertrophy and multiplication involve epidermal, mesophyll and vascular tissue. Cells over the vascular bundles form ridges, while those between are depressed into furrows (Thorne, 1961).

Goodey (1932) worked out on plants infected with different Anguina spp. A. agrostis was,located in the pith and in the outer parts of the cortex in red fusiform swellings of the host and in terminal rosettes of thickened leaves of Thymus vulgaris. A. pratensis made its way through root tissues, broken down cell walls, and fed on cell contents, brought about distortation of cortical tissue of roots. A. agropyronifloris invaded only

fertile florets of wheat grass forming elongated galls as a consequence of cell elongation rather than by cell division. A. Agrostis induced galls in Lolium rigidum. Galls typically developed in place of ovules, less commonly in place of stamens, and rarely on glume or rachis. The galls consisted of a wall several cell layers deep surrounding a central cavity. Cells adjacent to the cavity were modified, presumably to provide metabolites for development and reproduction of the nematodes. Cells at the surface remained unchanged, thus maintaining the structure of galls.

Norton and Sass (1966) showed that the only part of the inflorescence, of wheat-grass (Agropyron smithii) invaded by A. agropyronifloris was the undeveloped fertile ovary, prior to stamen enlargement. Invasion of the ovary occurred down to, but not including the procambium. Cells in the distal half of the undeveloped ovary became enlarged, vacuolate and loosely arranged. Stynes and Bird (1982) studied the development of galls induced by A. agrostis in ryegrass (Lolium rigidum). The galls which grew rapidly as nematodes developed, consisted of a wall several cell layers deep surrounding a central cavity. Cells adjacent to the cavity were modified, presumably to provide metabolites for development and reproduction of the nametodes, which completed a single generation before plant senescence occurred and the galls dried out. Cells at the surface were unchanged, thus maintaining the structure of galls.

3.3.3.3. Aphelenchoides Fischer, 1894.

The foliar leaf nematode, Aphelenchoides enters through stomata into leaf mesophyll and destroys tissues during feeding. Lesion become brown; usually appearing as angular sectors between large leaf veins. They also reach flower buds. Christie and Arndt, (1936) frequently found A. parietens associated with lesions on the underground parts of plants, especially on the hypocotyle of seedlings. Sometimes these nemas migrated beyond the diseased area penetrating apparently healthy cortical tissue, and were found coiled within cells or intercellular spaces.

Todd and Atkins (1958), washed root, culm, leaf and panicles of various plants, then stained and examined. No nematodes were observed within stained tissues, however, nematodes were recovered from leaf sheath washings confirming the ectoparasitic nature of this foliar nematode. A ritzema-bosi fed almost entirely ectoparasitically on the epidermal cells of embryonic leaves and the shoot apex. Outer 2 - 3 layers of cells were killed and a thick red stained layer was formed. The red stained layer inhibited growth and division of the underlying intact cells which became enlarged, vacuolated, contained little cytoplasm, and whose nuclei were large, reddish and granular with apparently normal nuclei. They sometimes penetrated through stomata into mesophyll tissue. Nematodes were often seen in substomatal cavities and the

accompanying guard cells were swollen causing the apertures to enlarge. Cavities in which many eggs were seen were most frequent in the spongy mesophyll especially around vascular bundles (Krusberg, 1961).

3.3.3.4. Rhadinaphelenchus Goodey, 1960

The coconut nematodes Rhadinaphelenchus spp. multiply rapidly within palms and enormous numbers invade all parts of the plant. A band of necrotic tissue appears in the lower portions of the stems. This band may be 3 cm wide encircling the stem 2.5 cm beneath the surface. Local areas of necrosis not forming a complete ring develop in the upper portions. The reddish color results from the death of cells upon which the nematodes have fed. The infection causes occlusion of xylem vessels (Dropkin, 1980).

Blair and Darling (1968) found R. cocophilus in ground parenchyma cells in stems, petioles and cortex of roots in the coconut palm. Nematodes were not confined to the discolored zone, but also occurred in smaller numbers intercellularly in the white parenchyma for a distance of 1.5 cm on the outside and 4 cm on the inside of the discolored tissue as well as in the meristematic region above the area of discrete lesions. In the region of discrete lesions, the nematode occurred intercellularly in the ground parenchyma of the stem. In cross-section, they sometimes appeared as heavily.....

Contd.

stained rounded dots, or they followed the shapes of intercellular spaces they occupied. In longitudinal section, they were intertwined between cells. Occasionally, they were seen neatly coiled in some cells and in large intercellular spaces. They also occurred intercellularly in the bundle sheath lying parallel to immature fibres, but not in the xylem nor phloem tissues.

3.3.3.5. Bursaphelenchus

Pine wood nematode is transmitted by a wood boring beetle from dead to uninfected trees of pines in Japan.

Yasuhara and Kiyohara (1972) found in Japanese red pine (Pinus densiflora) and black pine (Pinus thunbergii) infected with B. lignicolus that the nematode was present in both axial and radial resin canals. The resin canal paranchyma cells, epithelial cells, of nematode infested wood were conspicuously damaged and in many cases, they were completely destroyed. The resin canals were supposed to be the site of infestation and the epithelial cells the most likely feeding site. Myres (1982) found pine wood nematode in the axial resin canals of Japanese black, Scot, and southern white pine. The nematodes killed parenchyma rays, cambial layers and rays. Myres (1986) inoculated ten different species of pine with B. xylophilus and

observed that the nematodes migrated from the cortex through the radial rays or directly across the sieve cells of the phloem to the cambium. The nematodes moved vertically as well as circumferentially destroying the fusiform and ray initials and their derivatives. Nematodes also occurred between xylem and phloem.

4. Plan of Work

PLAN OF WORK

Syncytia or giant cells form-ation is must for the successful establishment of host-parasite relations in root-knot nematode infection. Regarding the formation and development of syncytia various hypotheses and explanations have been put forwarded by different workers. Many problems still remain unsolved and it is not precisely known whether giant cell formation and development are dependent on a continuous stimulus from the nematode or whether only an initial stimulus is required to trigger off cell reactions that lead to the formation of giant cell without further stimulus from the nematode. Mankau and Linford (1960) supported the former hypothesis but further experimental evidence is required to establish it.

To establish the correlations between the structural changes caused in the host tissue as a result of nematode infections and the effects on host plants depriving them of nutrients by disturbing their normal physiological function is also essential.

Sponge gourd (Luffa cylindrica L.) said to be indigenous to India, is a large climber, grown throughout India. Smooth, cylindrical, tender fruits of sponge gourd are used as vegetable. It is important not only as a vegetable but also as a medicine

A clear liquid, extracted from the stem is useful in respiratory complaints. Ripe fruit, after burning and pulverizing, is used as carminative and anthelmintic. Mature seeds are bitter, emetic and cathartic. Seed oil is used for skin affections. Apart from fungal and viral diseases, it is also attacked by root-knot nematodes. The galls formed, due to root-knot nematodes in the root are well developed and prominent. As far as histopathological studies of spong gourd roots, infected with root-knot nematodes, are concerned, the literature, as the review indicates, is silent. No work till now has been carried out on anatomical anamolies. Attempts, also have not been made to establish the effects of root-knot nematodes on anatomical, cytological and physiological changes of the phloem tissue. Therefore, it is proposed to investigate the histopathological changes, in complex tissues, with special emphasis on phloem, due to root-knot infection. Following aspects will be included in the proposed plan of work :

1. To study the effect of different inoculum levels of M. incognita on the development of giant cells in Luffa cylindrica roots.

2. To trace the different cytological changes leading to the formation of syncytia.

3. To study the damage caused by root-knot nematode infection to vascular tissues in L. cylindrica roots as a consequence of root-knot nematode infection.

4. To trace the histopathological changes, in roots leading to gall formation.

5. To study the correlation between extent of damage caused to vascular elements and reduction in growth of plants and physiological symptoms resulting from disturbed physiology of infected plant.

6. To study the difference in histopathological response of some varieties resistant and susceptible to root-knot nematode.

7. To study the effect of different ecological stresses on the formation of giant cell and development of gall.

5. Materials and Methods

MATERIALS AND METHODS

The different materials to be used and methods to be employed during the course of proposed experimental programme are generalized as follows :

5.1 Test Plants and Pathogens :

In the proposed plan of work sponge gourd (*Luffa cylindrica* L.) will be selected as test plant and root-knot nematode (*Meloidogyne incognita*) as test pathogen.

5.2 Collection of Inoculum of M. incognita :

Root-knot nematode infected roots will be collected from vegetable crop fields. Species of root-knot nematodes and races will be identified on the basis of the characteristic of perineal patterns and differential host test.

5.3 Pure Culturing and Maintenance of Inoculum :

A single eggmass of *M. incognita* will be surface sterilized in 1 : 500 solution of Chlorox (calcium hypochlorite) for five minutes and washed thrice in sterilized distilled water. The egg-mass will then be allowed to hatch in distilled water at

27°C. Egg-plant seedlings raised in 25 cm pots containing autoclaved soil will be inoculated with the larvae thus obtained. After about two months the soil of inoculated pots will be examined to ascertain the establishment of nematodes. Then subculturing will be done by inoculating new egg-plants with at least 15 egg masses obtained from pure culture in order to maintain sufficient inoculum throughout the course of investigation.

5.4. Inoculation of Nematodes :

Prior to inoculation the egg masses will be removed from the infected roots of egg plant and allowed to hatch (Stemerding, 1963). The counting of second stage juveniles of M. incognita will be done with the help of counting dish under the stereoscopic microscope. Three days after seedling emergence holes of 5 - 7 cm depth around the plants within a radius of 2 cm from the plant will be made, in which a counted number of nematode larvae will be transferred with the help of sterilized pipette. The holes will then be plugged with sterilized soil. Regular watering will be done to maintain the soil moisture till the termination of the experiment.

5.5. Histopathological Studies :

Inoculated seedlings will be uprooted carefully of the soil from the first until the sixth day, then every three days until

the 30th day and finally on the 40th day. The roots will be washed gently, but thoroughly to remove all soil particles adhering to them. Galled roots will be cut into one cm long pieces and processed as follows :

5.5.1. Fixation :

The roots collected from experimental pots will be fixed in FAA (Johansen, 1940). FAA (Formalin-aceto-alcohol) will be prepared as follows :

Ethanol 50%	90 ml
Formaline 37%	5 ml
Glacial acetic acid	5 ml

The roots will be placed in the fixative for a minimum period of 24 hr to several days, depending on its thickness. Material may also be stored in the fixative indefinitely.

5.5.2. Dehydration :

Dehydration is accomp-lished by moving the roots stepwise through increasingly higher concentrations of alcohols. Tertiary butyl-alcohol (TBA) dehydration schedule (Table 1) will be followed (Johansen, 1940).

TABLE - I

Step	% Alcohol	Time	Distilled water (ml)	95% Ethanol (ml)	100% Ethanol (ml)	100% TBA (ml)
1.	50	2 hr or more	50	40	0	10
2.	70	overnight	30	50	0	20
3.	85	1 - 2 hr	15	50	0	35
4.	95	1 - 2 hr	0	45	0	35
5.	100	1 - 3 hr	0	0	25	75
*6.	100	1 - 3 hr	0	0	0	100
7.	100	1 - 3 hr	0	0	0	100
8.	100	overnight	0	0	0	100

* TBA changes must be carried out in a warm place as it solidifies at 25.5°C.

5.5.3. Infiltration :

In this step, alcohols in the tissues will be replaced by paraffin so that the tissue is saturated with a pure solution of paraffin. When the TBA dehydration schedule is followed, the 100% TBA solution in step 8 will be replaced with a 1 : 1 mixture of 100% TBA and paraffin oil. The tissue will be allowed to remain in this solution for 1 hr or more, depending on its thickness. Shortly before the next step, another container will be filled ¾th of its volume with melted paraffin and allowed to solidify slightly. The roots from the TBA-paraffin oil mixture will then be placed on top of the solidified paraffin oil solution. This container will be placed uncovered in an oven set at slightly above the melting point of the paraffin. After 1-3 hr, the TBA - paraffin oil mixture will be poured off and replaced with pure melted paraffin wax, and kept in oven for about 3 hr. This step will be repeated at least once more.

5.5.4. Embedding :

The roots will be placed in metal base molds or folded paper. Molds will first be coated with a thin layer of glycerine and then liquid paraffin will be poured. Roots will be placed into the mold with heated foceps and additional melted paraffin will be added to fill the mold. Once the paraffin begins to

solidify over the top of the mold, the mold will be plunged into ice water and left there until solidification. After hardening, it will be cut into smaller blocks and trimmed. The blocks will be mounted on block holders.

5.5.5. Sectioning :

Transverse and longitudinal sections of 8 - 12 µm thickness of the roots will be cut serially with the help of rotary microtome. The paraffin ribbon thus obtained will be mounted on a clean glass slide with an amount of albumin and glycerine dissolved in water. The slides will be left overnight in an incubator at 40 ^{o}C to allow water to evaporate. These slides will then be kept at 60^{o}C for one hr to melt the paraffin.

5.5.6. Staining :

The process of staining will remove the paraffin from the sections and increase the contrast in the tissues. Staining will be done with Safranin and Fast Green combination (Table 2) (Sass, 1951). Then, slides will be removed from the xylene, the final step in a staining procedures and laid on a flat, absorbant surface. The mounting medium will be applied to the surface of the slide before evaporation of the xylene, and cover-slip will be lowered gradually over the slide.

TABLE - 2

Step	Solution	Time
1.	xylene	5 min
2.	absolute ethanol	5 min
3.	95% ethanol	5 min
4.	70% ethanol	5 min
5.	50% ethanol	5 min
6.	30% ethanol	5 min
7.	1% aqueous safranim O	1-12 hr
8.	rinse in tap water	
9.	30% ethanol	3 min
10.	50% ethanol	3 min
11.	70% ethanol	3 min
12.	95% ethanol	3 min
13.	0.1% fast green FCF in 95% ethanol	5-30 sec
14.	absolute ethanol	15 sec
15.	absolute ethanol	3 min
16.	xylene-absolute ethanol	5 min
17.	xylene	5 min
18.	sylene	5 min or longer

Finished slides will be left to dry for at least 24 hr at room temperature. The medium will harden better if the slides are held on a 60°C warming tray overnight. The slides will be examined under Laborlux - K compound microscope. Necessary photographs will be taken. To study the morphology of individual vascular elements namely xylem of the infected roots, they will be macerated in hot HNO_3, following the methods prescribed by Ghouse and Yunus (1972).

5.6. Physiological Studies :

To correlate the extent of damage to the vascular elements due to M. incognita infection and deficiency symptoms of nitrogen, phosphorus and potassium exhibited by the foliage, the seedling of spo-nge gourd will be grown in glazed pots having acid leached sand and fed with 25 ml of complete Long Ashton solution (Hewitt, 1966), the composition given in table 3.& 4.

TABLE - 3

Macronutrients	ppm			
KNO_3	K	156	N	57
$Ca(NO_3)_2$ Anhyd.	Ca	160	N	113
$MgSO_4 . 7H_2O$	Mg	36	S	48
NaH_2PO_4	Na	31	P	41

TABLE - 4

Micronutrients		ppm
Fe. Citrate. $3H_2O$	Fe	5.6
$MnSO_4$. $4H_sO$	Mn	0.55
$CuSO_4$. $5H_2O$	Cu	0.064
$ZnSO_4$. $7H_2O$	Zn	0.065
NaCl	Cl	3.5 Na 2.3
H_3BO_3	B	0.54
$(NH_4)_6$ Mo_7O_{24} $4H_2O$	Mo	0.048

Plants will be inoculated with different inoculum levels in log scale. The histopathology of infected roots will be done. Nitrogen, phosphorus and potassium present in the leaves of plants inoculated with different inoculum levels will be estimated and compared with the amounts in the leaves of uninoculated plants.

For the estimation of nitrogen and phosphorus 'Spectronic 20' (Bausch and Lamb) will be used. 'Systronix' flame-photometer

will be used for the estimation of potassium. Nitrogen will be estimated by Linder (1944) and phosphorus by Fiske and Subba Row (1925) method respectively. Potassium will be estimated directly from aliquot by flame-photometer.

5.6.1. Leaf Analysis for N, P, and K. :

Leaf analysis is an established practice for assessing the nutritional status of the plants (Lundegårdh, 1951). Fully mature and healthy leaves of dried samples will be powdered finely, and passed through a 72 mesh screen. The leaf powder will be kept at 70°C overnight before acid digestion according to the method of Linder (1944) that is briefly described below.

From each sample, 100 mg of the dried leaf powder will be carefully transferred to a 50.0 ml Kjeldahl flask and 2.0 ml of chemically pure sulphuric acid will be added. The flask will be kept for digestion for about two hr to allow complete reduction of nitrates present in the plant material, giving off dense white fumes until the contents turn black.

Flask will then be allowed to cool down for about 15 min, after which 0.5 ml of 30% hydrogen peroxide will be added dropwise and the solution will be heated again till its colour changes from black to light yellow. Heating will be continued for about

30 min. The flask will be cooled for 10 min and an additional amount of 3-4 drops of 30% hydrogen peroxide will be added, followed by gentle heating for another 15 min, to get a clear and colorless extract. At this stage, care will be taken in the addition of hyd-rogen peroxide because f it is added in excess there is a possibility that it would oxidise the ammonia in the absence of organic matter. The sulphuric acid-peroxide digested material will be diluted with double distilled water and will be transferred with 3 or 4 washings to a 100 ml volumetric flask and finally the volume will be made upto the mark. For the analysis of nitrogen, phosphorus and potassium, aliquots will be taken from these digested samples, the methods employed for this analysis are briefly summarized below :

Nitrogen :

Nitrogen content of the sample will be estimated according to the method of Linder (1944). A 10.0 ml aliquot of the digested material will be taken in a 50 ml volumetric flask. The excess of acid will be partially neutralized with 2.0 ml of 2.5 N sodium hydroxide and 1.0 ml of 10% sodium silicate will be added to prevent turbidity. Finally, the volume will be made upto the mark. A 5.0 ml aliquot of this solution will be taken in a 10 ml graduated test tube and 0.5 ml of Nessler's reagent will be added drop by drop, mixing thoroughly after each instalment. The volume will again be made upto the mark with the help of distilled water

and the contents will be allowed to stand for 5 min for maximum color development. The solution will be transferred to a colorimetric tube and the optical density will be measured at 525 nm on a colorimeter. A blank will be run with each set. The amount of nitrogen in the aliquot will be read from a calibration curve, obtained by using known dilution of a standard ammonium sulphate solution.

Phosphorus :

Total phosphorus in the sulphuric acid - peroxide digested solution will be determined by following the method of Fiske and Subba Row (1925). A 5.0 ml of aliquot will be taken in a 10.0 ml graduated tube and 1.0 ml molybdic acid (2.5% ammonium molybdate in 10N H_2SO_4) will be carefully added, followed by 0.4 ml of 1, 2, 4 - aminonaphthol - sulphonic acid, which will turn the contents blue. Distilled water will be added to make up the volume upto 10.0 ml and the solution will be allowed to stand for about 5 min after mixing thoroughly. It will be then transferred to a colorimetric tube and the optical density will be read at 620 nm on a colorimeter. A blank will be run side by side. A calibration curve will be prepared by using known dilution of a standard monobasic potassium phosphate solution.

Potassium :

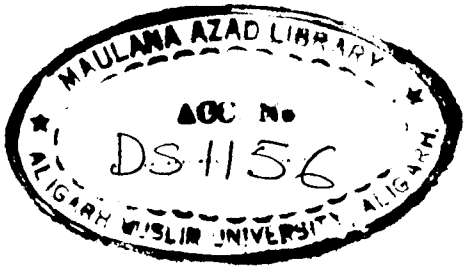
Potassium will be estimated using a flame photometer. A 1.0 ml aliquot will be taken and read at 768 mm with the help of potassium filter. A blank will be run for each determination. The readings will be compared with a calibration curve plotted for different dilutions of a standard potassium sulphate solution.

5.7. Studies on Effects of Soil Moisture and Soil Temperature :

To study the effect of different soil moisture and soil temperature levels on the histopathological changes in Luffa seedlings, plants will be grown at different soil moisture levels as described below and will be inoculated with M. incognita and infected roots will be processed for histopathological studies.

5.7.1 Soil Moisture :

In order to study the effect of different soil moisture levels on the histological changes, 10%, 15%, 20%, 25% and 30% moisture levels will be used. First of all the water holding capacity and moisture content of the soil to be used will be determined. Then the desired moisture levels will be maintained by adding requisite amount of water to the soil contained in pots. The surface of the pots will be covered with cellophane sheets in order to check the loss of water.



5.7.2. Soil Temperature :

Effect of soil temperature will be studied in the Wisconsin type temperature tanks. Seedlings of test plants will be transplanted in 15 cm clay pots having autoclaved soil and inoculated with nematodes. The pots will then be transferred to temperature tanks running at 10^{o}, 15^{o}, 20^{o}, 25^{o}, and 30^{0}C.

The data will be analysed statistically and will be incorporated in the form of a thesis for the award of Ph.D degree of the ALIGARH MUSLIM UNIVERSITY, ALIGARH.

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